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Effects of Gibberellic Acid, *N*-6-Benzylaminopurine, and Acetone on Pinyon *(Pinus edulis)* Germination

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Pinyon *(Pinus edulis)* seeds from two north-central Arizona sites were treated with gibberellic acid (GA_3), *N*-6-benzylaminopurine (BAP), or a combination of the two hormones in order to improve germination capacity and energy. The hormones were applied under vacuum using either water or acetone as a carrier. All hormone treatments were ineffective. Germination percentages for hormone treatments in water were similar to those of the controls. Hormones in acetone depressed germination; none of the acetone treatments reached 50% germination during the study period. Some possible explanations for the results are discussed.

Keywords: Pinyon, *Pinus edulis,* germination, gibberellic acid, cytokinin, BAP, acetone

Introduction

Pinyon-juniper woodlands cover approximately 47 million acres in the western United States (Evans 1988). These woodlands generally occur on relatively dry sites in the Southwest between 4,500 and 7,500 feet in elevation where annual precipitation can vary from 12 to 22 inches (Springfield 1976). Pinyon is a common species throughout most of the ecosystem, where it is usually associated with one or several juniper (*Juniperus* sp.) species.

Pinyon produce large edible seeds which are important food for wildlife, and in earlier times, for humans. Nut gathering is still a popular activity. *Pinus edulis* starts peak seed production when trees are between 75 and 100 years old (Gottfried 1987). Cones take 3 seasons to mature, and an average cone can contain between 10 and 20 wingless seeds. Good seed crops occur at 4- to 7-year intervals although more frequent crops can occur on better sites.

Although natural regeneration is the main goal of most silvicultural prescriptions, the interest in artificial regeneration of pinyon has been increasing. Planting of nursery-grown pinyon seedlings is currently being conducted to reclaim strip-mined areas and to rehabilitate critical habitat types which have been damaged by fire.

The most common procedure for breaking pine seed dormancy is to soak the seeds for 1 to 2 days and then to apply cold stratification at between 33°and 41° F for a period depending on the species and the nature of the seed dormancy (Krugman and Jenkinson 1974). Other techniques soak the seeds in water or treat them with organic or inorganic chemicals at various concentrations or time intervals, or combine chemical treatments with cold stratification.

The use of plant hormones such as gibberellic acid (GA_3) and cytokinins to stimulate germination is becoming more common. There are about 60 known gibberellins (Salisbury and Ross 1985), although GA_3 is the one most commonly used in seed germination work. It is generally believed that gibberellins from the embryo initiate germination by stimulating the production of α-amylase which hydrolyses starch in the endosperm. While several tree seed studies (Biswas et al. 1972; Bonner 1976; Heidmann 1981, 1986a; Jenkins 1961) have shown positive effects from the application of exogenous GA_3, Salisbury and Ross (1985) indicate that GA_3 has a less dramatic effect on food reserve mobilization in dicots and gymnosperms than in cereal grains. They indicate that gibberellins enhance cell elongation so that the radicle can push through the endosperm and seed coat.

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Cytokinins are another group of hormones involved with germination. They can be used to promote germination, often in situations where GA_3 alone is ineffective (Khan 1975). Khan (1975) indicates that cytokinins are essential to reduce the effects of abscisic acid (ABA) which inhibits germination by preventing α-amylase production. This allows the gibberellins to be effective. Gibberellic acid and *N*-6-benzylaminopurine (BAP), a cytokinin, together can completely reverse the influence of ABA in ponderosa pine (*P. ponderosa*) (Heidmann 1986a).

The penetration of hormones through the seed coat is critical. Bonner (1976) reports that germination is enhanced by increasing the seed-soaking times and concentrations of GA_3. It is often necessary to mix plant hormones with other chemicals to facilitate hormone entry through the seed coat. This appears necessary because of the relatively high molecular weights of some hormones, such as GA_3 (molecular weight of 346.38). Acetone has been used as a carrier in several studies concerned with field crop species (Milborrow 1963, Tao and Khan 1974) without an adverse effect on germination. However, Heidmann (1986b) reports that soaking ponderosa pine seeds in acetone, even for 1 hour, repressed germination. He also noted that the effects of acetone could be reversed when hormones were included in the solution (Heidmann 1986a).

Few studies have addressed seed dormancy in pinyon or methods of enhancing or accelerating the germination process. Krugman and Jenkinson (1974) recommended 0 to 60 days of cold stratification for stored seeds. Gottfried and Heidmann (1986) found that some seed lots of Arizona seed benefited more from 60-day cold stratification than from 30-day stratification, but overall, the two periods gave similar results. Riffle and Springfield (1968) indicated that pinyon seeds treated with hydrogen peroxide germinated twice as fast as untreated seeds, while Gottfried and Heidmann (1986) reported variable results from their tests using six combinations of various hydrogen peroxide concentrations and soaking times.

The main objective of the current study was to evaluate the effects of gibberellic acid, *N*-6-benzylaminopurine, and a combination of the two hormones on pinyon germination capacity and energy, and to evaluate the impacts of acetone and water as carriers for the hormones.

Methods

Pinyon seeds were collected from two areas on the Colorado Plateau of north-central Arizona in October 1986. One site is in the vicinity of Cedar Ranch, approximately 28 miles northwest of Flagstaff, within the Coconino National Forest. This site is adjacent to US Highway 180 and is at about 7,000 feet elevation. The second site is at Tusayan, several miles south of the Grand Canyon airport, at an elevation of 6,900 feet. This area, which is within the Kaibab National Forest, is about 33 miles northwest of the Cedar Ranch site. Seeds collected from individual trees were stored separately to allow an evaluation of variability within each stand and comparison of results between stands. Seeds were collected from these two general areas in 1983 for the previous study by Gottfried and Heidmann (1986).

Seeds were stored in a cool locker at approximately 38° F. Prior to the study, seeds from three trees from each site were floated in n-pentane to remove unfilled seeds. Seeds were rinsed in distilled water following the test. In March, 1987, subsets of 25 seeds from each tree were also germinated in a Hoffman[2] germinator programmed for 12 hours of light at 76° F and 12 hours of dark at 60° F. The three lots of Cedar Ranch seeds had an average germination capacity, with standard error, of 98.7% ± 1.3%; and an average germination energy, which is measured here to be the number of days to achieve 50% germination, of 12.3 ± 0.9 days. The Tusayan seeds had an average capacity of 93.3% ± 1.3% and had a germination energy equal to that of Cedar Ranch.

There were six hormone treatments and a control treatment, where the seeds were only rinsed in distilled water. The two hormones were prepared as 10^{-4} molar solutions. Each solution was tested on 100 seed subsets from each of the 6 trees. Beakers of seeds and solution were placed in a vacuum chamber, and vacuum was applied and maintained for 15 minutes after the first bubbles were observed from the seeds. The bubbles indicated that gases were being drawn from the seeds. The vacuum was then released and the seeds were allowed to stand for another 15 minutes; hormones should enter the seeds during this period. The next step was to remove the seeds from the solution and rinse them twice in distilled water.

The treated seeds were then transported to a greenhouse where they were divided into 50 seed lots and seeded into large pots containing a mixture of equal parts of peat moss and perlite. Seeds were covered with about one-quarter inch of mixture. A randomized design was used to locate pots within the greenhouse. A seedling was considered germinated when its cotyledons were free of the soil. Pots were inspected almost daily and germination recorded; they were all watered, as necessary, to maintain uniform moisture conditions. Germinated seedlings were removed from the pots.

The experiment was begun in May 1987, and continued for an 85-day period. Germination capacity and energy were analyzed by multivariate analysis of variance procedures. Data from individual locations were evaluated by one-way analysis of variance and Tukey's

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[2] The use of trade and company names is for the benefit of the reader; such use does not constitute an official endorsement or approval of any service or product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.

multiple comparison procedures. Data were tested for equal variances using Bartlett's test, and normality was evaluated by normal and detrended normal plots. A 150-day default value was used for treatments that did not achieve a 50% germination value by the end of the study. Statistical significance was indicated by values at or above the 5% probability level.

Results

Germination Capacity

The percent germination data showed a distinct dichotomy with seeds treated with hormones in water behaving similarly to the controls, while seeds treated with hormones in acetone showed depressed germination relative to the controls or to the water carrier group (fig. 1). The treatments produced similar responses from the Cedar Ranch and Tusayan seed lots (fig.1). The average germination for all water carrier treatments, combining seed sources, was 92.3% ± 1.1%, while the average for the six acetone treatments was 14.8% ± 2.6%. The germination percentages for the Cedar Ranch and Tusayan control lots were 96.1% ± 2.2% and 94.3% ± 2.4%, respectively. Germination within carrier group or location was not affected by hormone treatments; GA_3, BAP, and the combination of GA_3 and BAP produced similar germination capacities (fig. 1). Hormones in the water carrier did not improve germination relative to the untreated control treatment.

Germination Energy

None of the seeds treated with hormones in acetone achieved 50% germination within the 85-day study period while all control seeds and those treated with hormones in water did meet this criterion. The three hormone treatments, disregarding the influences of the carriers, were ineffective in stimulating the speed of germination. Treatments combining hormones and water produced results similar to controls (fig. 2). The analysis did indicate significant differences in germination energy between the Cedar Ranch and Tusayan locations (fig. 2). This difference is indicated by values for the control seed lots; Cedar Ranch controls reached 50% germination within 23.7 ± 1.2 days, while Tusayan controls reached this level within 27.7 ± 0.9 days. One-way analyses of variance for the data for each location still showed the effects of carrier and the ineffectiveness of hormones.

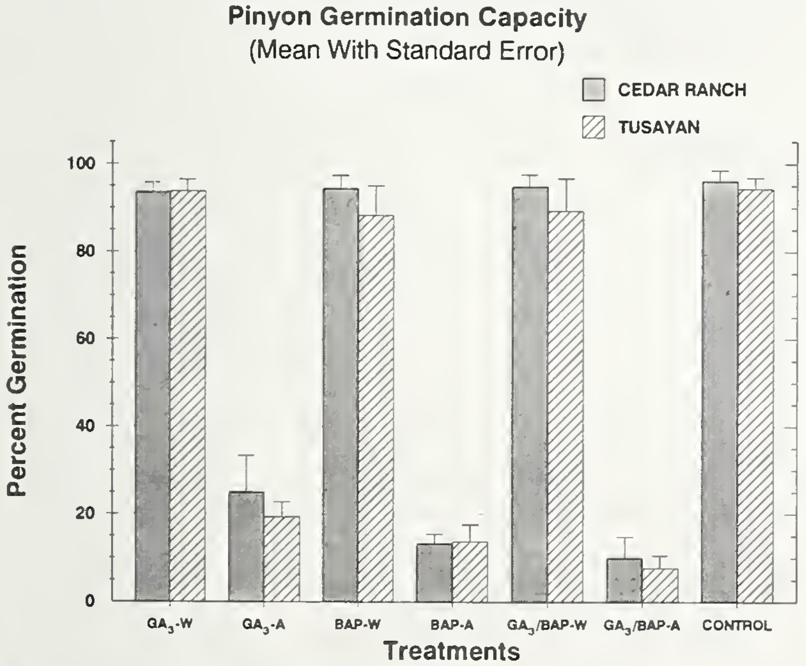

Figure 1.—Gibberellic acid (GA_3), *N*-6-benzylaminopurine (BAP), and a combination of GA_3 and BAP, in the acetone carrier (a) depressed pinyon germination capacity for both Cedar Ranch and Tusayan seed lots. Hormones in the water carrier (w) were ineffective in increasing germination.

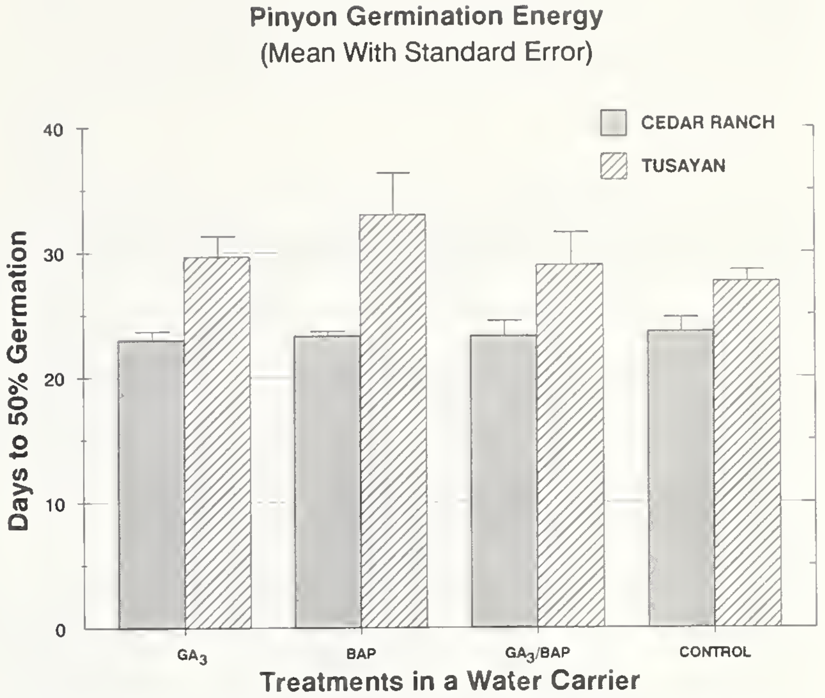

Figure 2.—Germination energy, based on the number of days to achieve 50% germination, for the hormone in water carrier treatments and for the controls. None of the pinyon seeds treated with the acetone carrier had 50% germination.

Discussion

Gibberellic acid, BAP, and a combination of the two hormones, in water or acetone, were ineffective in increasing the germination capacity and germination energy of two pinyon seed sources from central Arizona. Heidmann (1986a) also found that hormones applied under vacuum in a water carrier did not improve germination of ponderosa pine seeds. These results are surprising. Whether GA_3 influences amylase production or enhances cell elongation, it has been generally shown to aid pine seed germination. Biswas et al. (1972) reported that soaking unstratified loblolly pine (*P. taeda*) seeds in 100 ppm of GA_3 improved germination capacity but did not affect speed of germination. Heidmann (1981) found that GA_3 improved germination energy of ponderosa pine under a range of temperature conditions. Jenkins (1961) also found that soaking eastern white pine (*P.*

strobus) seeds in 100 ppm or 500 ppm for 24 and 48 hours increased percent germination over soaking seeds in water without the hormone. Heidmann (1986a) showed the effectiveness of cytokinin and of a combination of cytokinin and GA_3, in acetone, on ponderosa pine germination. However, Biswas et al. (1972) found that kinetin did not improve germination of unstratified loblolly pine seed but that it was beneficial at concentrations of 10 ppm on seeds that had been stratified for 21 days.

There are, however, several possible reasons why hormones were ineffective. One explanation is that endogenous hormone levels were already sufficient to initiate and sustain germination, as Bonner (1976) indicated, and that the added hormones were superfluous. Treated seeds did not germinate better than untreated seeds. The results of the current study, as well as those of Gottfried and Heidmann (1986) indicate that germination success of untreated pinyon seeds is normally high; it ranged from 94% to 96% here and averaged 92% in the previous study. Even cold stratification did not increase the germination capacity of pinyon; it only accelerated the process. It is possible that seed lots with lower natural germination characteristics or older seed would have reacted differently. Another possible factor is that the chemicals were not reaching the embryo because they were not penetrating through the seed coat. Chemicals must penetrate both the inner layer of the seed coat, which is the major site of seed dormancy in some pines (for example, *P. monticola,* Hoff 1987), and the hard outer seed coat. Heidmann (1986a) believes that the distilled water carrier applied under vacuum did not carry enough hormones into the seeds to influence germination. Most tests have shown that soaking for longer periods of time seemed to enhance the effects of GA_3 (Biswas et al. 1972, Jenkins 1961). It is possible that forcing the chemicals into the seeds with the vacuum technique was less successful than the more passive soaking procedure.

These hypotheses could be tested by assaying hormone levels of untreated and treated seeds. Hormones tagged with radioactive tracers also could be used to determine if hormones were reaching the seed embryos. Another possibility is that the vacuum technique was forcing endogenous hormones out of the seed as well as forcing exogenous hormones into them, resulting in no gain in hormone levels. A careful assay of the solution following treatment possibly could verify this hypothesis.

Hormone concentrations have been demonstrated to affect the amount of hormones entering the seeds and subsequent germination during soaking experiments (Biswas et al. 1972, Tao and Khan 1974). Most experiments with tree seeds have been successful with GA_3 concentrations between 100 and 1000 ppm (Biswas et al. 1972, Bonner 1976, Jenkins 1961); the 365 ppm concentration used on the pinyon seeds was within this range and should have been effective. Ideally, other concentrations of hormones should be tested on pinyon.

Another clear result of this study was that acetone depressed pinyon germination. This is contrary to the findings of Milborrow (1963) and of Tao and Khan (1974) who reported that acetone did not depress germination of various field and horticultural crop species, such as barley (*Herodeum* sp.), squash *(Cucurbita maxina),* and pear *(Pyrus communis).* Milborrow (1963) reported that seeds could be soaked in acetone for up to 6 months without any adverse effects on germination or subsequent seedling growth. However, Heidmann (1986a, b) found that ponderosa pine seeds in acetone gave variable and inconsistent results but that even a 1-hour soak could suppress germination. The percent germination of seeds treated with acetone alone was half of that for the controls; however, the effects of acetone could be moderated by the presence of hormones (Heidmann 1986a). The current study did not evaluate acetone alone but the beneficial effects of the added hormones were not apparent.

The reasons for the current results, as well as of those of Heidmann (1986b), are not clear. It is possible that the acetone treatments could have leached the endogenous hormones from the seeds, and that there was little retention of the exogenous compounds, thus causing a net decline in gibberellin and cytokinin levels. Milborrow (1963) indicated that acetone could be used to remove germination-inhibiting compounds from seeds as well as to add enhancing substances. It is just as likely that some of the enhancing hormones could also be removed. This possibility could be confirmed if higher levels of hormone were found in the solutions after treatment. It is also possible that acetone adversely affects seed enzymes or has more adverse effects on pine embryos than on embryos of cereals and lettuce (*Lactuca* sp.). The fact that some seeds germinated could indicate genetic differences among seeds or the effects of local conditions within the beaker during treatment.

The results of this study showing the ineffectiveness of the hormones and the adverse effects of acetone should be applied to other seed sources of pinyon with caution. Although the same treatments may produce similar results, it would be difficult to make very general statements about a species that has such a wide geographic and climatic range without further testing. It is logical to assume a great deal of genetic variability in *Pinus edulis.* Even our two seed sources from the Colorado Plateau of central Arizona showed some basic differences in germination energy while reacting similarly to the chemical treatments. Gottfried and Heidmann (1986) found differences in pinyon germination energy among five Arizona seed lots, including seeds from Cedar Ranch and Tusayan. Seeds from the higher elevations and more northern sites germinated quicker than seeds from more common sites. Other seed sources may also have lower inherent germination capacities and energies than those tested. The hormone and carrier treatments were applied to some pinyon seeds from Prescott, Arizona which had a germination capacity of

86.3% ± 6.7% and a germination energy of 42.0 ± 2.0 days. This area, south of the Colorado Plateau at 5,100 feet in elevation, contains a pinyon population whose taxonomy is still unclear; trees in the same stand can contain one needle per fascicle, two needles per fascicle, or both types of needles. Results showed the same depression of germination for acetone treatments and the same ineffectiveness of hormones in water as found for the two Colorado Plateau seed lots. However, variability was high in this population, and larger quantities of seed from more trees would need to be tested to confirm these observations. The fact that Prescott seed showed a similar reaction to the treatments does, however, tend to support the general results.

Conclusions

Gibberellic acid and BAP applied individually or combined, in either a water or acetone carrier, did not enhance the germination capacity or energy of *Pinus edulis* from sites in central Arizona. Acetone depressed germination regardless of the hormone treatment. It appears that acetone should be used with caution in treating seeds of any pine species. Although it is difficult to make general conclusions from a limited sample, it appears that further testing of additional seed sources and other concentrations of hormones are necessary before making any general conclusions about the usefulness of GA_3 and BAP.

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